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RT-PCR cycle threshold (Ct) values predicting COVID-19 patients' outcome

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Abstract
Aim: Clinical presentation of COVID-19 ranges from asymptomatic to fatal cases. Therefore, predictability of prognosis gains importance in managing the disease. The aim of this study is to investigate the relation between RT-PCR cycle threshold (Ct) values and the clinical severity of COVID-19 infection.
Material and Methods: A retrospective study was conducted among 1224 COVID-19 patients. A scoring system, which is designed by the World Health Organization was used to classify patients by means of their clinical status.
Results: The cut-off for Ct value in ROC curves was 21.52 at the point, when the COVID-19 patient clinic is shifting from ambulatory to hospitalized (79.7% sensitivity, 69% specificity). A significant weak positive correlation was found between age and WHO Score ($r= .238$ $p<0.01$) and a significant weak negative correlation was found between Ct value and WHO Score ($r= -.068$ $p<0.05$) in COVID-19 patients.
Discussion: Patients with lower RT-PCR Ct values were more likely to go through the disease more severely due to higher virulence. Reporting of numerical Ct values may help clinicians in terms of prognosis.

Keywords
PCR, Cycle Threshold, COVID-19, SARS CoV-2

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Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2), a novel viral agent that belongs to the Coronaviridae family, is an enveloped RNA virus, which is transmitted from person to person via respiratory route [1,2]. Coronavirus Disease 2019 (COVID-19) first emerged in Wuhan, China in December 2019. As of the date of submission of this manuscript, globally more than 414 million cases and over 5.8 million deaths from COVID-19 have been reported to the WHO (available at: https://covid19.who.int). WHO classifies COVID-19 cases as suspected, probable and confirmed. This classification is based on clinical and epidemiological criteria [available at: https://apps.who.int/iris/handle/10665/337834 and https://apps.who.int/iris/handle/10665/332196). The clinical spectrum of COVID-19 changes from ambulatory mild cases to patients in the intensive care unit. Real time polymerase chain reaction (RT-PCR) is used to detect the presence of the virus in nasopharyngeal and oropharyngeal samples, through the identification of one or two gene regions of the virus (one gene for screening, two genes for confirmation) [3]. The cycle threshold is characterized as the number of cycles needed for the fluorescent signal to pass the threshold in order to be detected. Ct levels are conversely related to the quantity of target nucleic acid in the sample [4]. In addition to RT-PCR assays, medical imaging techniques as well as routine clinical chemistry tests are critical for both diagnosis and predict clinical prognosis of COVID-19 patients [5]. In this paper, it was aimed to show the relationship between Ct values and COVID-19 patients' clinical outcome. Reporting of numerical Ct values may help clinicians regarding the prognosis of COVID-19 patients.

Material and Methods

Data Collection

The study was planned and conducted using a tertiary care hospital. This retrospective cohort study included COVID-19 patients admitted to this hospital from September 15, 2020 to February 12, 2021 with a positive SARS CoV-2 PCR test. For the PCR test nasopharyngeal samples were collected at the time of hospital admission of symptomatic patients. Therefore, sample collection and clinical evaluation were performed simultaneously. Of the 1392 patients with positive SARS-CoV-2 PCR tests, the ones with missing any of baseline characteristics were excluded from the study. Ct values could be obtained from 1224 patients (53.1% males, 46.9% females). Patients included in this study were aged between 18 and 80. The study has been approved by the COVID-19 Scientific Research Evaluation Commission of the Ministery of Health of the Republic of Turkey and by the local institutional ethics committee (Protocol number: E-22481095-020-451).

PCR Testing

The Senteligo SARS-CoV-2 (COVID-19) qPCR Detection Kit Protocol, which is optimized for HealForce X960 real-time PCR (qPCR) analyzer (Eryigit, Ankara, Turkey) was followed, the analyzer provides 35 cycles upon the measure of PCR product. A single one-step reverse transcription qPCR test was used for amplification of the targets (N1, N2 and RnaseP) by FAM, HEX and Cy5 labelled probes, respectively. A negative, positive and internal control were used for each run.

Study Design

Patient information regarding Ct values of PCR tests and hospital admission data were obtained from the electronic health records of the hospital. The data is evaluated and filtered depending on the date, age, clinical status and the relevant test results. The clinical status of the patients was demonstrated with a scoring system, which was designed by WHO for the classification of COVID-19 patients (8). In this scoring system, classification depends on clinical conditions and is scored from uninfected to death between 0 to 8.

Statistical Analysis

All statistical analyses were performed in NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) Software. Descriptive statistics (medians, standard deviation, frequency, minimum and maximum values) and distribution of the data were reported using the Shapiro-Wilk Test. To estimate associations between two independent groups the Mann-Whitney U Test was used. Spearman's Correlation Analysis was performed to determine the association between two quantitative data groups. The significance level was evaluated as $p<0.01$ and $p<0.05$.

Results

From September 15, 2020 to February 12, 2021, 17.531 viral screening PCR tests were done in the hospital among patients with COVID-19 suspicion. The study population flow chart is shown in Figure 1.

The number of the patients grouped by their clinical status using WHO scores were as follows: 1051 patients for a score of 2, 106 patients for a score of 4, 13 patients for a score of 5, 5 patients for a score of 7, 49 patients for a score of 8. The difference between clinical identifications of score 1 and 2 can be interpreted subjectively thus patients in these groups were all scored as 2 in order to unify present data correctly. Since applied additional treatment supports to the patients were not fully detailed in the records, all patients who were hospitalized in COVID-19 ward were scored as 4, and patients who were intubated were scored as 7, regardless of receiving additional organ support.

The mean age of patients was 41.91±16.8 years ranging from 18 to 80; 85.8% (n=1050) of the study population were classified as COVID-19 ambulatory, whereas 14.2% (n=174) of all the patients admitted to the hospital. Among the patients who were admitted to the hospital, 113 of them stayed in the COVID-19 ward, whereas 61 of them were admitted to the ICU. Forty-six (75.4%) of 61 patients who needed intensive care were intubated. During the hospitalization process, 49 (4%) of them died and 125 (10.21%) of them were discharged. The mean age of the survivors (n=1175) was 40.91±15.97 years, when the mean age of the non-survivors (n=49) was 65.86±19.52 years. Ct values changed from 7.5 to 34.18 with a mean of 24.3±3.57. There was no statistically significant difference in Ct values between ambulatory, COVID-19 service admission and ICU admission in comparison to sex and age (Table 1).

A significant weak positive Spearman's correlation was found between age and WHO Score ($r= .238$ $p<0.01$) versus significant weak negative Spearman's correlation between Ct Value and WHO Score ($r= -.068$ $p<0.05$). ROC analysis based on the Ct

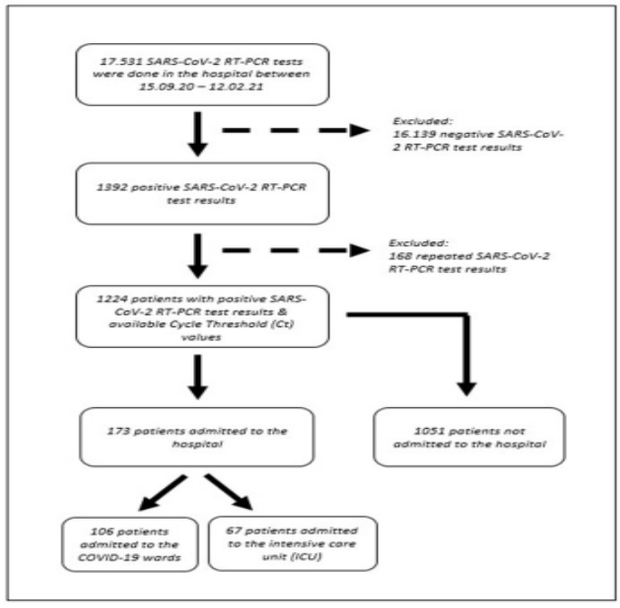

Figure 1. Study Population Flow Chart

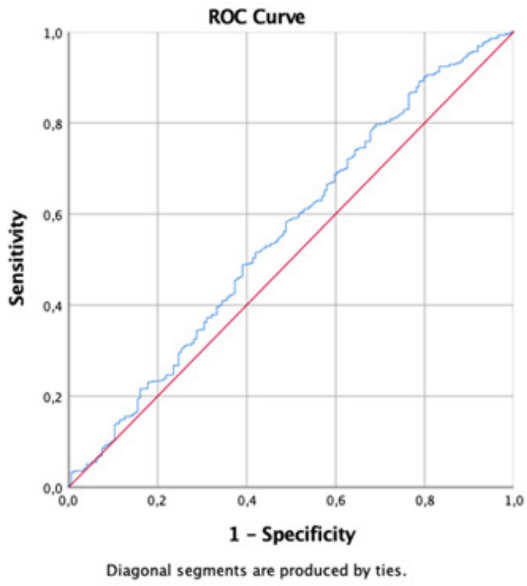

Figure 2. Sensitivity and Specificity ROC Curve for Hospital Admission by Cycle Threshold (Ct) Values

values regarding hospital admission accounted for 55.7% as area under the curve for evaluating the severity of the disease (Figure 2).

The Ct cut-off value was found as 21.52 in ROC analysis to evaluate COVID-19 patients' hospitalization needs (ambulatory or hospitalized). This cut-off value had 79.7% sensitivity and 69% specificity.

Discussion

The uncertainty of the diagnosis and discharge criteria of COVID-19 creates a burden on health systems. In this research, it was aimed to show the relationship between Ct values and COVID-19 patients' clinical outcomes. Reporting numerical Ct values may help clinicians regarding the prognosis of COVID-19 patients. In Turkey, there is no sufficient research based on this topic.

According to our findings, the cut-off value based on the Ct value for the assessment of the COVID-19 patients' hospitalization needs was <21.52. Below the cut-off value (<21.52), higher WHO score points were observed. It was aimed to establish the cut-off value by using ROC analysis so that this cut-off value may give an idea of the patients' clinical outcome either ambulatory or hospitalized on the day of the SARS CoV-2 PCR test result becomes positive. In a prospective cohort study, which was conducted by Tanacan et al., they investigated the relationship between Ct values and obstetric complications in COVID-19, and the cut-off value was found to be 22.9 [6]. In addition, Cerutti et al. categorized values as ≤25, 25–28, 28–30, 30–35, >35 to compare mean Ct values of symptomatic SARS CoV-2 antigen test positive and negative patients. They observed significantly lower Ct values for PCR-positive/antigen positive samples with a mean value of 22.3, and higher Ct values for PCR-positive/antigen negative samples with a mean value of 32.1 [7]. We did not perform antigen testing because of the low sensitivity and specificity in comparison to PCR. Furthermore, Miller et al. claimed that lower Ct values are indicators for more severe progression of the disease, however they did not define a precise cut-off value with acceptable sensitivity and/or specificity for triage with an AUC value of 65.37% [3].

According to Walker et al., low Ct values are associated with higher viral load in COVID-19 patients [8]. As supported by Lyngse et al., low Ct values indicate higher viral load, which

Table 1. Mean and Minimum/Maximum Cycle Threshold (Ct) Values Classified by Sex and Age

		Ambulatory (n=1050)	COVID-19 Service Admission (n=113)	Intensive Care Unit Admission (n=61)
		Mean ±Sd	Mean±Sd	Mean±Sd
		Min-Max (Median)	Min-Max (Median)	Min-Max (Median)
Cycle Threshold (Ct) Value	Male (n=650)	24.24±3.48 8.04-34.18 (24.31)	23.99±3.71 14.91-33.12 (24.02)	22.98±4.59 7.5-30.77 (22.66)
	Female (n=574)	24.62±3.49 11.43-34.06 (24.82)	23.24±4.25 12.09-30.87 (24.28)	23.97±3.55 18.16-30.7 (23.55)
p value (sex)		0.093	0.626	0.422
Cycle Threshold (Ct) Value	18-35y (n=509)	24.45±3.43 8.04-33.52 (24.68)	23.92±3.6 14.95-30.87 (23.9)	25.06±3.19 20.42-30.77 (25.23)
	36-65y (n=580)	24.32±3.56 9.62-34.18 (24.19)	23.04±3.93 12.09-30.8 (23.69)	23±3.84 16.22-30.47 (22.88)
	>65y (n=135)	24.9±3.39 11.43-30.84 (25.21)	25.16±4.1 16.44-33.12 (25.47)	23.23±4.47 7.5-30.7 (23.47)
p value (age)		0.172	0.158	0.359

is also associated with an increased rate of SARS CoV-2 transmission [9]. Miranda et al. suggest standardization of Ct values with a formula to improve the interpretation of viral load in the samples [10]. Choudhuri et al. conducted a retrospective study including 1044 SARS CoV-2 positive patients and they demonstrated that Ct values are independent predictors of patient mortality [11]. In a systematic review of eighteen studies, a correlation between Ct values and disease severity was reported by eleven of them, mentioning that the low Ct value would l lead to more serious consequences. There was a significant relationship between Ct value and disease severity among hospitalized COVID-19 patients (73% of the investigations) [9]. In this study, it was observed that ambulatory patients had higher Ct values compared to the patients who were hospitalized in the COVID-19 service (WHO score of 4) and intensive care unit (WHO score of 5 and 7) (P=.001; P<.01). Even though Zhao et al. report that Ct values are significantly associated with viral load among survivors and non-survivors, we did not find any correlation between patient survival and Ct values (P>.05) [12]. The difference of results can be explained by the limitations of RT-PCR testing. Pre-analytical and analytical factors such as sampling procedures, accuracy of the detection kits, specimen obtaining techniques, whether the specimen is taken before or after the symptoms started can affect PCR results [13]. Ct value of men and women who received mechanical ventilation support were 22.98 (4.59) and 23.97 (3.55), respectively, and statistically significant correlation was not found between Ct values and sex of the ICU admitted patients (P=0.422). According to Jin et al. although both sexes were equally susceptible to the coronavirus infection, fatal outcomes were more likely to be seen among male patients [14]. In this present study, the majority of cases were men but we did not show any significant relationship between clinical severity and the sex of the patients (P>.05). In another study, males were more involved in the severe group in the hospitalization period, however no statistically significant difference between Ct values and the sex was found [15]. Moreover, we did not find a significant correlation between Ct values and sex (P>.05). In this retrospective cohort, the data examined did not show any difference in the distribution of Ct values among different age categories, which is in parallel with the study conducted by Ade et al. [16]. The mortality rate in our study was 4% with an increased rate in patients older than 40 years of age. The ages of the patients who died were significantly higher than those who survived (P<.01). Moreover, patients with older age were more likely to have higher WHO scores (P<.01). As supported by Miller et. al, a tendency toward mortality was significantly higher for the elderly and the ones who were hospitalized [3]. It has been shown that comorbidities lead the COVID-19 patient into a vicious infectious cycle and are closely associated with morbidity and mortality [17]. In our study, we did not evaluate patients in terms of comorbidities.

Among patients who required mechanical ventilation, mortality rate was more than 70%. Richardson et al. reported that the mortality rate was 76.4% in the same patient group aged between 18 and 65 likewise our results [18]. According to the COVID-19 Diagnostic Laboratories Quality Management Guide of Turkish Ministry of Health, SARS CoV-2 PCR test results with Ct value of less than 26 are regarded as high positive, 26 to 30 moderate positive and more than 30 low positivite by method with 35 cycles (available at: https://shgmkalitedb.saglik.gov.tr/Eklenti/37841/0/covid-19-kalite-rehber-03072020-sooonnnnpdf.pdf). However, in Turkey SARS CoV-2 RT-PCR test results are reported qualitatively as negative or positive to the clinicians by public health management system. Furthermore in addition to clinical symptoms, numerical Ct values can be used to predict the outcome of COVID-19 patients. Additional research on this topic can help to enhance the understanding of the clinical course. Giving CT results in addition to the qualitative result can guide clinicians about the course of the disease.

This study has several limitations. The data included in the study were restricted to electronic records of the hospital. We took into account only the first PCR test result of patients at the time of admission to the hospital. Serial testing of PCR in patients who were treated in the COVID service and ICU can be evaluated with prospective studies to make a comparison within the groups. A scoring system was used to classify patients for a better understanding of their clinical status. The scores were changing from 1 to 8 as expressed previously. Unfortunately, applied additional treatment supports were not fully detailed in the health records. Hence, the modified version of the scoring did not contain scores 3 and 6. Furthermore, nonhospitalized COVID-19 patients were classified with the WHO score of 2 because the distinction between scores 1 and 2 can be misinterpreted, so a score 1 was not used. In this study, comorbidities of the patients were not taken into consideration while evaluating their clinical status. Underlying diseases should be included in the assessment of patients' medical conditions for a better understanding of the related case.

Conclusion

The result of the presented data supports that the interpretation of Ct values at the time of admission with SARS CoV-2 RT-PCR positivity, can lead clinicians to make better predictions about the clinical course. Patients with lower Ct values are particularly at higher risk for poor clinical outcomes, whereas patients with higher Ct values usually experience a mild form of the disease. To predict clinical outcome, the cut-off value in ROC curves with the highest sensitivity/specificity was found to be 21.52, which could shed a light on further adjustments of related guidelines and have an impact on precautions taken during the management of COVID-19 cases.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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